PATENT

Attorney Docket No. 11823-002630

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| In re application of: | Examiner: J. Burke |
| CARY L. QUEEN ET AL. | Art Unit: 1642 |
| Application No.: 08/484,537 | |
| Filed: June 7, 1995 | |
| For: IMPROVED HUMANIZED IMMUNOGLOBULINS | |

DECLARATION OF CARY L. QUEEN

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Cary L. Queen, declare and state as follows:

1. I received my Ph.D. in 1975 from the University of California at Berkeley. I am an author of 50 scientific publications, many of which report on research in molecular immunology, including humanized immunoglobulins. I am now Senior Vice President and Vice President, Research at Protein Design Labs, Inc. In this capacity, one of my primary responsibilities is to oversee the company's antibody humanization program. A copy of my curriculum vitae is attached as Exhibit 1.

2. I am a named inventor of the subject patent application. I have read the Office Actions dated Nov. 18, 1997 and April 29, 1999, as well as the references cited therein.

3. The Examiner takes the position that the specification has not enabled determining which sequences are 65% or 70% identical, because sequence identity has no common meaning within the art, since the scoring of gaps when comparing one sequence to another introduces uncertainty as to the percent of similarity. Although this may be correct with respect to certain protein sequences, it is not correct with respect to immunoglobulin heavy chain variable region framework sequences, the only type of sequence being compared in the claims.

4. In fact, at the time of filing of the application, and at the current time, anyone of skill in the art would normally align two immunoglobulin heavy chain variable region framework sequences according to the numbering system of Kabat. In what follows, I will refer to immunoglobulin variable regions sequences as Ig sequences. Dr. Kabat and colleagues maintain a comprehensive, frequently updated list of the known Ig sequences, published from 1976 - 1991 in Kabat et al., Sequences of Proteins of Immunological Interest (U.S. Department of Health and Human Services) and now available as a computer data base.

5. Kabat uses a numbering system for Ig sequences, which assigns each amino acid of each sequence a number. The numbering system is used to align such sequences in a direct and unambiguous way: amino acids with the same number designation are aligned with each other. All the hundreds of Ig sequences in the Kabat volumes are aligned with each other (after grouping by chain type, species and subgroup) in this way. For example, the first page of the list of Ig heavy chain sequences in the 1991 edition of Kabat et al., displaying 22 human heavy chain sequences of subgroup I aligned by Kabat numbering, is attached here as Exhibit 2.

6. Because the Kabat data base is the universally recognized compendia of Ig sequences, and provides a multitude of examples of Ig sequences aligned by Kabat numbering and in no other way, one of skill in the art would naturally align any two heavy chain variable region framework sequences by Kabat numbering - it would probably not even occur to the skilled person to do otherwise.

7. There is a second important reason that two heavy chain framework sequences would inevitably be aligned by Kabat numbering. Namely, as explained in more detail below, framework sequences almost never have gaps with respect to each other (for any one type of chain, such as heavy chains). And without gaps, two such sequences can only sensibly be aligned in one way, which is precisely the way specified by Kabat numbering. Indeed, it is because Ig framework sequences do not have gaps that it is possible to assign Kabat numbering to any such sequence in the first place.

8. To determine whether framework sequences have gaps when aligned with each other, I examined the Ig sequences listed in Kabat et al. (1991). I observed that there were extremely few gaps, as shown by missing amino acids, in the frameworks of the sequences. For example, visual

examination of the 22 heavy chain sequences in Exhibit 2 shows that there is not a single gap in the frameworks of these sequences.[1] In the few cases where there is a missing amino acid, this is likely due to incomplete sequence information rather than to an actual gap, since Kabat et al. states, "In all instances residues considered uncertain by the authors have not been included in the table." (p. xiv). In contrast, there are obviously many gaps in the CDRs of the Ig sequences.

9. In further reviewing Kabat et al., I noted that at positions in the Ig sequences where gaps are possible, the Kabat numbering scheme uses lettered numbers. For example, examination of Exhibit 2 shows that the Kabat numbering system progresses ... 34, 35, 35A, 35B, 36, 37 .... This allows one Ig sequence to have amino acids at positions 35A and 35B whereas another sequence does not, i.e., for there to be a gap, while amino acids 35, 36, 37, etc. are still aligned according to Kabat numbering. I noted that lettered numbers are used in each of the 3 CDRs to allow gaps there.

10. However, I further noted that there are no lettered numbers in the parts of the heavy chain framework regions labeled FR1, FR2 and FR4, indicating that Kabat found no gaps there. There are lettered numbers 82A, 82B and 82C in FR3 (see Exhibit 2), so gaps are potentially possible in that part of the framework. However, in fact almost all heavy chain Ig sequences in Kabat et al. actually have amino acids at all those positions (e.g., see again Exhibit 2), e.g., except for a few rabbit Ig sequences (Kabat et al., 1991, p. 514-517), so there are few actual gaps. I do not believe those few gaps would have presented to one of skill any difficulty or ambiguity in aligning heavy chain framework sequences, both because of the extreme rarity of the gaps, and because their position is well-defined within the sequence - between amino acids 82 and 83.

11. As an independent method of determining whether heavy chain framework sequences have gaps when aligned with each other, I examined an extensive listing of human heavy chain germline sequences[2] (Fig. 2(a) of Tomlinson et al., J. Mol. Biol. 227: 776 - 798), which is believed to include much of the human germline repertoire. A copy of this reference is provided as Exhibit 3. The authors align the framework sequences according to Kabat numbering, again indicating the universality of this method. Visual inspection of this listing shows that there is not a single gap in the framework region (FR1, FR2 and FR3) of the 83 sequences listed.[3]

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful false statments and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statments may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Dated: July 12, 1999

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[1] Several of the sequences are missing amino acids at their end, but this is because the amino acids were unknown due to incomplete sequence information, rather than because of gaps.

[2] Because the sequences are unrearranged germline sequences, they do not include the last part of the framework sequence, FR4, which is encoded separately by the J regions.

[3] Several of the sequences are missing amino acids at their end (and in one case at the beginning), but this is believed to be due to incomplete sequence information rather than actual gaps.